392 POSTER

Influence of wortmannin on non-homologous DNA end joining in human normal and cancer cells

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DNA double-strand breaks (DSBs) are the most genotoxic lesions. If they are not repaired they may lead to cell death, if mis-repaired they may result in mutations or cancer transformation. Human cells repair DSBs mainly via the non-homologous end joining (NHEJ) pathway. DNA-PKcs kinase activity plays an important role in this pathway. We have employed an in vitro assay to study the role of DNA-PKcs in NHEJ reactions. In this method, fluorescent dye allowed for direct visualization of rejoined linearized plasmids by human cell extracts. We used the DNA-PKcs kinase inhibitor wortmannin to measure the rejoining sensitivity of cell extracts to the drug. Our findings demonstrate that rejoining by human normal and cancer cells is relatively insensitive to wortmannin under the conditions of our assay. Moreover, DNA-PKcs immunodepletion resulted in only a modest reduction of end joining. In conclusion, our data suggest that under specific defined in vitro NHEJ reaction conditions, the presence of DNA-PKcs is not stringently required for production of end joined products.

393 POSTER

Determination of some biochemical effects associated with boron neutron capture therapy in experimental hepatomas

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Background: Boron neutron capture therapy (BNCT) is based on the selective delivery of boron-10 (^{10}B) to tumor cells. Following irradiation with low-energy neutrons, nuclear capture and fission reactions occur that produce He^{2+} and Li^{3+} particles. The effectiveness of BNCT is dependent upon the amount of ^{10}B deliver per cell. Approximately 10^9 ^{10}B atoms per tumor cell are necessary to produce four to five particles per cell, but studies of radiation-induced apoptosis suggest that BNCT also may be cytotoxic via other mechanisms so that the required number of ^{10}B atoms actually may be less. The aim of our paper is to find new biochemical mechanisms such us the oxidative destruction involved in tumoral cell cytotoxicities.
Materials and Methods: RS1 hepatoma-bearing rats were given single i.p. injection of 30 mg ml^{-1} of a BPA: fructose 1.0:1.1 molar solution. Mice were euthanized 1, 3, or 6 h after the injection. Tumor, blood, and liver were assayed for boron biodistribution and oxidative stress parameters Than the tissues was irradiated 2700 seconds with a 1.472×10^5 n/cm^2 epitermal fluency beam.
Results: Our results show preferential capture of BPA at tumoral level with a maximum value at 3 hours after the administration, the number of 10B atoms calculated in one gram of tumoral tissue is ranging between 10^{12} and 10^{17} atoms, the highest value of BPA internalization in tumoral cells is in the range of 20–40 μg. The lipid peroxides level measured in blood after the BPA administration is increasing two times at the hepatoma bearing rats than in normal control, also the caeruloplasmin Cu-oxidase activity growth from 168 I.U. to 330 I.U., the albuminic thiol-groups are decreasing from 267 μmol/l at 107 μmol/l
Conclusions The BPA administration possibly induce methabolic pathways wich involves the oxigen consumption, and after the irradiations the cytotoxicities is done by oxigen free radical production The biochemical parameters of oxidative stress can be used in monitoring the evolution of hepatoma, the modifications after BPA administration and possible the irradiation effects

394 POSTER

Inhibition of thymidine phosphorylase decreases tumour aggressiveness but reduces chemosensitivity in liver fluke related cholangiocarcinoma

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Background: Liver fluke related cholangiocarcinoma (CCA), the most common malignancy in the Northeast Thailand, is an important public health problem because the incidence and the fatality rate are high. Thymidine phosphorylase (TP) gene has been shown to be amplified (53.8%) in CCA of our previous report suggesting that TP may play an important role in carcinogenesis or pathogenesis of CCA.
Materials and Methods: We evaluated the role of TP by RNA interference (RNAi) using small interfering RNA (siRNA) directed against the human TP mRNAs in KKU-M139 CCA cell line, which has naturally high level of endogenous TP. TP-siRNA knockdown cells were tested for functions in vitro.
Results: siRNA targeting of TP dramatically impaired the expression up to $87.1 \pm 0.49\%$ of mRNA and $72.5 \pm 3.2\%$ of TP protein compared with those of control. We have demonstrated that TP depletion by siRNA reduced TP-induced proliferation and migration of KKU-M139, and suppressed TP activity on inducing migration and tube formation of human umbilical vein endothelial cells (HUVECs). siRNAs also interfered the ability of TP on resistance to hypoxia-induced apoptosis but not UV-induced apoptosis of KKU-M139. On the other hand, suppression of TP reduced the response of KKU-M139 to 5-fluorouracil (5-FU) chemotherapy. However, combination of siRNA knockdown and UV exposure significantly decreased the concentration of 5-FU required to inhibit cell proliferation compared with that of siRNA alone.
Conclusions: We suggest that it is useful to examine expression level of TP in tumour tissues for selecting patients who are likely to response to 5-FU. Since TP increases tumor aggressiveness and there are several chemotherapeutic drugs of choice, inhibiting TP activity by such tools (siRNA) may be a good benefit for improving the poor prognosis of cancer patients who show high expressions of TP.

395 POSTER

Quantitative prediction of cancer drug efficacy by AKT and ERK1/2 down regulation in pancreatic cancer

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Cancer drugs are increasingly designed to target specific cell-signaling pathways. However, the pathways governing apoptosis in mammalian cancer cells are complex, and the pro- and antiapoptotic permutations are related to cell viability and resistance to cancer drugs according to species, cell types and also since they can be activated at different points. Therefore, it is hard to predict the best treatment for a particular tumor. Here, we examined the key enzymes quantitatively working as activation or inhibition by various cancer drugs among individual patients. As cancer cells, we used highly malignant human-derived pancreatic cancer line, MIA PaCa-2, PANC-1 and Suit-2 because pancreatic cancer cure is unusual with cancer recurrence as metastatic disease in many cases after removal surgery of the primary tumor. For cancer drugs, a recombinant humanized anti-HER2 antibody (Herceptin) as a practically used example and plant-derived phenolic compounds of quercetin, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one and trans-resveratrol, 5-[(1E)-2-(4-hydroxyphenyl)-ethenyl]-1,3-benzenediol to prevent cancers as daily digested food examples, respectively. First, we examined the receptor expression on cell membrane and the different level of expression was observed. Secondary, we cultured cells with various concentrations of cancer drugs for 48 h and observed the concentration dependent drug efficacy together with the different magnitude among cell lines as cell viability which related to caspase-3 activity implying apoptosis. Thirdly, we assayed enzymes after 24 h of cell culture with a given cancer drug and found the positive linear relationships between relative activity of AKT protein phosphorylation [pS473] and cell viability as $r = 0.941$, and between relative activity of both dual phosphorylation ERK2 [pTpY185/187] and ERK1 [pTpY202/204] and cell viability as $r = 0.959$ by ELISA using all cell lines and cancer drugs, respectively. These results suggest that the down regulation of both MAPK and AKT/PI-3K pathways quantitatively relates to cancer drug efficacy